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LOUIS PASTEUR AND THE ECOLOGY OF ANTHRAX

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by

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INTRODUCTION

Anthrax has been known as a scourge of livestock since antiquity. The identification of the bacillus causing the disease is less than a century past. Robert Koch, in 1876, reported the cultivation of the organism of anthrax, and that matter infected with anthrax preserved under varying conditions retained its pathogenicity for many years. It is now known that Bacillus anthracis (Cohn, 1872,) forms spores within the walls of the vegetative organism; these spores have an increased resistance to heat, chemicals, ageing, dryness and other adverse factors, in contrast to non-sporulating organisms causing infectious diseases. The readiness with which B. anthracis could be cultivated artifically, and the perplexity of the metabolism of pathogenic organisms which form both capsules and spores has made it a favored organism for laboratory study. Anthrax in livestock is accepted as an infectious disease which originates from the soil, and very little of the laboratory effort has been directed at correlation with the soil environment.

The hydrogen ion concentration was shown by S. H. Whitworth in 1924 to influence the growth and survival of B. anthracis. This study, and epidemiological data collected by C. D. Stein provided a basis for a soil-capability map by G. B. Van Ness and Stein in 1956. A real test of the soil-capability hypothesis came in the Oklahoma-Kansas anthrax outbreak of 1957.

More than six hundred premises near the Grand River in eastern Oklahoma and Kansas experienced a serious epidemic of anthrax. Although anthrax had occurred before in the area historically associated with the Sedalia Cattle Trail, such an epidemic was considered possible only because of a combination of climate and soil. The soil-capability hypothesis called for anthrax to occur on soils of calcareous origin, and not on shale soils of the area. Outbreaks were associated with soils having depressions, ponds, or flooded areas on which there was a browning of grass or the drying of an organic film on lime rocks or organic soils. Pastures not showing such "incubator areas" had no losses from anthrax. In 1958, the weather was unsuitable for the formation of incubator areas, and anthrax was not a problem.

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Since that time, the "incubator area" concept has been accepted and applied in endemic areas, but the idea that anthrax spores vegetate in the environment before there are infections of livestock has run head-on against the persistent spore theory.

It is enough to recognize the tenacity of B. anthracis spores in feed, animal byproducts, wool, hair, and bristles. These products are dry, which avoids spoilage and undesirable changes in quality. When the spores are subjected to moisture and nutrients, they may vegetate into a viable organism. If the environment and biological competition favor the organism, an increased capability to form more spores will follow. It is this increase in spore count which is fundamental to the "incubator area" concept. As either the vegetative or spore form may be infective, the biological opportunity to increase brings about an increased opportunity to infect.

The persistent spore theory rejects the idea of vegetation outside the animal. Fundamental to the rule that a specialized parasitic form has lost the capability to exist in ecological competition, the persistent spore theory may have spilled over to include many other microbiological pathogens. The effect is to cause the land owner to believe that once anthrax spores contaminates his land, the threat of further losses at any time can lower the value of land and affect livestock management.

LOUIS PASTEUR'S CONTRIBUTION TO THE ECOLOGY OF ANTHRAX

The revolution of microbiological thought in Pasteur's time centered on the rejection of spontaneous generation. The differentiation of many anthrax-like organisms which occur naturally in soil was a matter for future study. The most common of these is Bacillus cereus and B. megaterium intermediates. These anthracoid organisms create many technical problems related to the study of B. anthracis in the soil today.

Pasteur observed that earthworms could well be a factor in spreading anthrax. That factor alone, without considering the total ecology of the earthworm environment, provides an element of public hysteria associated with anthrax. A knowledgable reader today can appreciate what Pasteur observed, without losing sight of the anthracoid organisms found in the earthworm environment.

Since the remarks by Pasteur in 1880 are even more ecologically oriented than many current articles on anthrax, it is expedient to provide an English translation of the whole report.

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The following is the English translation[2/] of --
EXPERIMENTAL PATHOLOGY - On the etiology of anthrax; by M. PASTEUR, with the collaboration of Messrs. Chamberland and Roux

(From: Comptes Rendus des Seances de l'Academie des Sciences, 1880)

One of the most deadly diseases of domestic animals is generally known by the name of anthrax. Most of the departements of France have to suffer from its ravages, some of them to a lesser, others to a greater extent. There are Departements where the annual losses are counted by the millions: one of them is the Departement of the Eure-et-Loir. Out of the numerous flocks of sheep raised there, there is perhaps not even one that is not hit every year. A farmer considers himself fortunate, and does not even pay any attention to the disease, when not more than two or three percent of the animals making up his flock are killed by it. All countries know this scourge. In Russia it is so disastrous at times that it is called there the "Siberian pest".

Where does this disease come from? How does it spread? Could not an exact knowledge of its etiology lead to prophylactic measures that could be applied easily and might be capable of stamping out this frightful disease rapidly? These are the questions that I intend to answer, and to that end I have asked two young scientists to join me in this endeavor; they are full of enthusiasm and - like myself - deeply interested in the great problems raised by the study of the infectious disease. These men are Messrs. Chamberland and Roux.

It has long been believed that anthrax originated spontaneously due to the effect of various fortuitous causes: the nature of the land, of the water and of the feed as well as the breeding and raising (fattening) methods; all kinds of attempts have been made to explain its spontaneous existence; but, since the work done by Messrs. **Davaine** and Delafond in France and by Pollender and Brauell in Germany has called attention to the presence of a microscopic parasite in the blood of the animals which perished by that disease, since rigorous investigations have refuted the hypothesis of the spontaneous generation of these microscopic beings, and finally, since the effects of fermentation have been connected with the microbes, we have gradually become used to the idea that the animals affected by anthrax might pick up the germs of the disease, i.e. the germs of the parasite, in the outside world, and that there is never any spontaneous origin of the disease in the proper sense of the word. This opinion was supported also when, in 1876, Dr. Kock of Breslau had shown that the bacteridium in its vibrionic or bacillary form could be converted into true germ corpuscles or spores.

2/ Translated commercially - French to English.

Two years ago, I had the privilege of submitting to the Minister of Agriculture and to the President of the General Council of the Eure-et-Loir a project pertaining to investigations on the etiology of anthrax; they received it with eagerness. I also have the good fortune of meeting M. Maunoury, Mayor of the small village of St. Germain which is located a few miles from Chartres; he proved to be an enlightened farmer who was willing to permit me to keep a small flock of sheep on one of the fields of his farm under the conditions that are generally applied in the Beauce for keeping sheep in the fold in the open air. In addition, the Director of Agriculture obligingly put two apprentice-herdsmen of the school of Rambouillet at our disposal for the surveillance and feeding of the animals.

Our tests began during the first days of August 1878. At the outset, they consisted in feeding certain lots of sheep lucerne grass (alfalfa) that had been wetted with artificial cultures of anthrax bacteridia saturated with the parasite and with its germs. Without going into details, which will be published elsewhere, I shall summarize our first results by making the following points:

Despite the immense numbers of spores of bacteridia that had been ingested by all the sheep of one certain lot, many of them did not die although they often showed clear symptoms of the disease; others, a smaller number, died after presenting all symptoms of spontaneous anthrax, and following a period of incubation amounting to as many as 18 days, even though during the last stages of life the disease presents almost fulminant features such as have been frequently indicated by those who observed it, and which have caused the belief that the period of incubation is of a very short duration.

The mortality may be increased by adding to the feed, which has been contaminated with the germs of the parasite, pricking objects, particularly the pointed ends of the leaves of dried thistles, and most of all, the beards from the heads of barley that have been cut into small fragments of length of 0.01 m, more or less.

It was very important to find out, whether the autopsy of the animals which perished under these conditions presented any lesions that would be similar to those observed in animals that die spontaneously in the stables or among the flocks that are kept in the fold in the open air. In both cases, the lesions are identical, and their nature makes it possible to arrive at the conclusion that the disease begins in the mouth or in the opening of the throat. Our first findings of the nature were obtained on August 18, on the basis of autopsies that were carried out before our eyes by M. Bontet, Jr., and by Mr. Vinsot, a young veterinary trainee who had graduated from the School of Alfort, and who assisted us with great application during the entire period of the tests undertaken at St. Germain.

From that time on, the idea which motivated our investigations, viz. to make sure that the animals which die of anthrax spontaneously in the Departement of Eure-et-Loir, have been infected by a contagion due to the spores of the anthrax bacteridia found in their feed, became established most firmly in our minds.

There remains the problem of the origin of the germs of the bacteridia. When we reject any idea of the spontaneous generation of the parasite, it will be natural for us to concentrate all our attention right from the start on the animals which are buried in the ground.

This is what takes place whenever an animal dies spontaneously of anthrax: when a place for flaying and cutting up the carcasses is nearby, the body is taken there. When such a place is too far away, or when the animal is not very valuable, as is the case with sheep, a hole is dug right on the spot in the same field where the animals perished or, when it perished at the stable, in a field close to the farm. The hole is 50 cm, 60 cm, or 1 m deep, and the animal is buried in it and covered with earth. What will then happen in the hole, and will there be any possibility of the germs of the disease spreading from it? Certain persons will reply in the negative, as appears from the exact tests undertaken by Dr. Davaine which show that the animal which has suffered from anthrax is no longer able to transmit the disease after its putrefaction. Numerous experiments have been carried out, even very recently, by one of the learned professors of the School of Alfort, who is one of the great advocates of the spontaneous nature of all diseases. He arrived at the following conclusion: "The water saturated with anthrax-contaminated blood and with remains of the spleen, the molds originating in the stratification of the sand, of the ground, of the excrement brought from Chartres together with the remains of the carcasses have never caused (by inoculation) even the slightest manifestation of an anthrax-like nature" (Colin, Bulletin of the Academy of Medicine, 1879); but, in this case, it is necessary to take into account the difficulties of the investigation and Mr. Colin has not recognized these difficulties by any means.

It is a difficult problem as such to take earth from the fields of the Beauce and to demonstrate in it corpuscles having a diameter of one or two thousandths of one millimeter, and being capable of producing anthrax in animals by means of their inoculation. However, with the aid of appropriate washing operations, and by making use of the contagious capabilities of these germ-corpuscles on the rabbit and guinea-pig species, the matter would be easy, if these corpuscles of the anthracis parasite were the only ones found in the ground. But, the ground conceals an infinite number of microscopic germs and of various species, the cultures of which in vivo or in vitro are harmful to one another. I have called the attention of the Academy to these fights for existence among microscopic beings at several ocasions in the course of the last twenty years; moreover, in order to separate from the ground the anthrax bacteridia which it may contain in their germ state, we have to have recourse to special methods which are frequently very delicate in application: effect of the air or a vacuum, changes in the culture media, influence of more or less elevated temperatures all of which vary with the nature of the various germs are means which must be applied in order to prevent one germ from masking the presence of another. Any method used in a rough investigation is irrevocably condemned to impotence, and the negative results prove nothing except that the bacteridium did not come to the fore under the conditions of the experimental set-up used. The main argument invoked by the knowledgeable professor of Alfort in order to support the negative results of his numerous inoculations is the assertion that the anthrax disappears in the carcass of an animal that died of

anthrax at the moment of its putrefaction [3]. This assertion is correct and it was well known by the knackers even before Dr. Davaine provided a confirmation of that fact. Frequently, I have been assured by the knacker-men whom I observed as they handled animals that had died of anthrax, and whom I warned of the danger which they encountered, that the danger had disappeared when the animal was in an "advance" stage, and that there was reason for concern only as long as it was still warm. Even though that assertion is not exact when taken literally, it betrays nevertheless the existence of the fact in question. As soon as the baceridium, in its filiform state, is deprived of the contact with the air, e.g. as soon as it is plunged into a vacuum or into carbonic acid gas (carbon dioxide), it tends to become reabsorbed in the form of very fine, dead and inoffensive granulations. The putrefaction places it exactly into these conditions of the breaking-up of its tissue. Its germ-corpuscles do not undergo that effect and are preserved, as has first been demonstrated by Dr. Kock (Koch). However, that may be since at the moment of its death, the animal harbors the parasite only in its filiform stage, there can be no doubt that the putrefaction destroys it there in its entirety.

If we did not go any further than that opinion and applied it to the facts of nature in an absolute manner, we would have only an incomplete view of the truth.

Let us look at the burying of the carcass of a cow, a horse, or a sheep that has died of anthrax. Even if the animals are not cut up, is it not possible that a smaller or larger quantity of blood will spill outside the body? Is it not a common feature of the disease that at the moment of death blood will flow out of the nose and mouth, and that the urine is frequently tinged with blood? As a consequence, and we might well say, in all cases, the earth around the carcass will be contaminated by the blood. Moreover, several days are required, before the bacteridium dissolves into inoffensive granulations, due to the protective effect of the gases lacking in free oxygen and freed by the putrefaction; and during that time excessive bloating of the carcass will cause fluids to pour out from its interior through all the natural orifices, or in addition through tears in the skin and tissue. The blood and the substances that are mixed in that way with the surrounding aerated earth are no longer in a state of putrefaction, but much rather in that of a culture that is inducive to the formation of the germs of the bacteridium. Let us ask, however, for an immediate experimental confirmation of these preconceived opinions.

We added anthracic blood to the earth that had been wetted with yeast water or with urine at the temperatures prevailing during the summer, and at the temperatures which the fermentation of the carcasses is bound to maintain around them, as in a heap of manure. In less than twenty-four hours, a

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[3] The behavior of the anthrax organism in the decomposing carcass is well described by Pasteur. The same can be applied to experiments by Mr. Colin, although the intent was to support spontaneous generation. We recognize today the difficulties, if not the impossibilities of finding B. anthracis in the soil environment, even after serious losses have occurred on a particular pasture.

multiplication and resolution into germ-corpuscles of the bactericidia brought in by means of the blood took place. Subsequently, these germ corpuscles were found to be in the stage of their latent existence, ready to germinate and capable of transmitting anthrax, not only after months of staying in the ground, but even after years.

But in the case described, we still have to do with laboratory tests only. It is necessary to find out what happens in the open field with all the alternative conditions of dryness, humidity, and cultivation. For that reason, in August 1878, we buried in a garden on the farm of M. Maunoury, a sheep of his flock which had died spontaneously of anthrax, after an autopsy had been performed.

Ten months, and again fourteen months later, we took earth samples from the hole and it was easy to determine the presence of germ-corpuscles of the bacteridium, and to cause anthrax and death in guinea pigs by inoculation. Moreover, and this circumstance deserves the most careful attention, this same search for the germs was carried out successfully in the earth of the surface of the hole, even though that earth had not been moved in the meantime. Finally, our observations were extended to the earth of the ditches in the Jura mountains, where cows which had died of anthrax had been duried in a depth of 2 meters during the month of July 1878. Two years later, i.e. recently, we collected earth samples from the surface and extracted from them deposits that easily caused anthrax. In this period comprising the last two years, samples of this earth taken from the surface of the holes presented anthrax-provoking properties. Finally, we recognized that the germs of the surface of the earth covering the buried animals is found again after all operations of cultivating and harvesting; these later observations extended to hhe earth from our fields on the farm of M. Maunoury. On the other hand, the ground further away from the ditches did not cause anthrax.

I would not be surprised if at this moment, doubts about the exactness of the preceding facts arose in the minds of the Academy. It will be said that the earth, which is such a strong filtering agent, would permit the germs of microscopic beings to rise to its surface - according to these observations!

Such doubts might attach themselves even to the results of experiments and observations which M. Joubert and I have published earlier. We announced that the waters of springs which flow out of the ground even at an inconsiderable depth, are free of all germs to such an extent that they are not able to fertilize even those fluids that are most susceptible of being affected. Such waters, however, are lower than the grounds which the waters of the rain traverse continuously sometimes for centuries; the effect of these waters must constantly tend toward moving downward the finest particles of the earth that are located above these springs. The springs remain perfectly pure for ever despite the conditions that are propitious for their contamination; that is a clear proof that a certain thickness of earth will stop all solid particles, even the smallest ones. What is the difference from the conditions and results of the experiments which I have just reported, since here, on the contrary, we have to do with microscopic germs that are supposed to start from deep down and rise to the surface, i.e. in a direction opposing the flow of the rain water, and up to great heights! We are faced with a puzzle.

The Academy will be greatly surprised to hear its solution. Perhaps they will even be moved by the thought that the theory of germs which has just been born as far as experimental investigations are concerned, has held in store such unexpected revelations as far as science and its application are concerned. As a matter of fact, the ground worms are the carriers of the germs, and they are the ones that bring the terrible parasite from the depths of its burial back to the surface of the earth. The germs of anthrax together with a great number of other germs are found in the small cylinders of earth consisting of fine earth particles which the worms bring up to the surface of the earth and deposit on it after the morning dew or after rain. It is easy to perform a direct test to demonstrate this: place some worms into ground into which spores of bacteridia have been mixed, and have them live there; then open their bodies after a few days, observing all possible precautions, so as to extract from them the earth cylinders which fill their intestinal tract, and you will find the anthrax spores there in great numbers. It is quite evident that, when the loose ground of the surface of the holes containing the carcasses of anthracic animals contains anthrax germs - and it frequently does, in great numbers - these germs originate in the breaking up of the small excrement cylinders of the worms by the rain. The dust from this broken-up earth spreads to the plants close to the ground, and it is in that way that the animals will come upon the anthrax germs in their fold (pen) and in certain types of their feed. These germs will infect them just as was the case in our experiments in which we transmitted anthrax by contaminating the lucerne-grass (alfalfa) directly. What perspectives for the mind are opened up by these results as far as the possible influence of the earth on the etiology of diseases, the possible danger of the earth in cemeteries and the usefulness of cremation are concerned!

Do the worms not bring to the surface of the earth other germs which might not be less harmless to the worms that those of anthrax, but which would nevertheless be carriers of specific animal diseases? As a matter of fact, they are full of all kinds of germs at all times, and the anthrax germs are found there always in combination with the germs of putrefaction and of the septicemias.

And now, what of the prophylaxis against anthrax? Is it not indicated as a matter of course? It will be necessary to make an effort at never burying the animals in fields that are intended either for growing forage or keeping sheep in the fold. In all cases, when ever possible, we will have to select either sandy terrains or calcareous[4/] soils for burying them, which should, however, be very poor, only slightly humid and easily desiccated, or in one word, unsuitable for earth-worms to live in. The eminent present Director of Agriculture, M. Tisserand, told me recently that anthrax is unknown in the region of the Savarts of the Champagne. Shall we not have to attribute that to the fact that in these barren soils, just as in those of the Camp de Chalons, for example, the thickness of the arable soil ranges only from 0.15 to 0.20 m, and that that soil rests upon a bank of chalk where the worms are unable to live? In

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4/ Rocky soils, even calcereous in nature, are unsuitable for anthrax, because of their tendency to be well drained or unproductive of vegetation. The French distribution in 1880 on soils formed from calcareous clays, and not on soils of schist or granite origin is in agreement with that in the United States today.

such a terrain, the burying of an anthracic animal will produce a great number of germs which will remain in the deeper regions of the soil and will be unable to do any harm, due to the absence of worms in the soil.

It would be desirable that a careful statistical survey establish for various countries the relation between the places with and without anthrax and the nature of the soil as far as it is favorable or unfavorable to the presence of earthworms in the ground. M. Magne, a Member of the Academy of Medicine, has assured me that the regions in the Aveyron, where we find anthrax, have a calcareous clay soil, and the parts where anthrax is unknown, have a schistose or granitic soil. Moreover, I have herd that the earthworms find it difficult to survive in the latter.

I make bold to end this paper by asserting that - if the farmers so wish - anthrax will soon be a thing of the past for their animals and for their herdsmen, as well as for the butchers and the tanners in the cities, since anthrax and the malignant pustule are never spontaneous, since anthrax exists only where it has been "stored" and where its germs are spread with the unconscious complicity of the earthworms; since - by way of conclusion - anthrax will disappear within a few years from any place where the causes that keep it going will not find any support."

Upon the motion of M. Thenard, the Academy decides that the Memorandum of M. Pasteur will be transmitted to the Minister of Agriculture and Trade.

Subdivision of grazing areas by fences has created an unusual opportunity to study the relationship of the environment to the genesis of anthrax. Although anthrax may have an incubation period of several weeks, it is usually possible to relate infection to environment. The peculiar browning by flooding of vegetation in depressions is one form of "incubator area" associated with anthrax.

Fig. 1 -- On this pasture with the brown grass "incubator area" at the edge of the dry pond, eight cattle died of anthrax. Death occurred between October 7 and 9, 1963. The cattle were placed on the pasture October 1, 1963 and deaths occurred October 7 to 9. Previous loss of two cows on July 28 was not diagnosed but the herdsman who attended the autopsy developed a lesion diagnosed as anthrax on his wrist.

First losses from anthrax may be diagnosed as weed poisoning, lightning stroke, or bloat. Anthrax and anaerobic infections due to Clostridium are still confusing the diagnosis of livestock losses. The environment for anthrax also may become an environment for mortality from Clostridium infections.

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Fig. 2 -- This cow was diagnosed as dead of Clostridium infections, despite previous losses from anthrax on the same pasture.

Fig. 3 -- An environment for anaerobic Clostridium losses may occur concurrent with anthrax. It is a black mud pond without browning of vegetation. After a diagnosis of anthrax, an inspection should be made of the environment and vaccination against anaerobic infections may be indicated. Sometimes the circumstances do not justify vaccination against anthrax.

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